

First records of Croaking Gourami, *Trichopsis vittata* (Cuvier, 1831) (Teleostei: Osphronemidae), from Myanmar and Bangladesh

Michael Norén,¹ Sven O. Kullander,¹ Md. Mizanur Rahman², Abdur Rob Mollah²

1 Department of Zoology, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05, Sweden. **2** Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh.

Corresponding author: Michael Norén, michael.noren@nrm.se

Abstract

The Croaking Gourami, *Trichopsis vittata* (Cuvier, 1831), is native to Southeast Asia and Sundaland, with introductions reported from USA, Philippines and India. The species was found by us in Myanmar (1997 and 2013), and Bangladesh (2014 and 2016). DNA analysis supports the view that *T. vittata* is a species complex. Specimens from Bangladesh, Myanmar and the European aquarium trade are the same genotype as specimens from Thailand, possibly corresponding to *Trichopsis harrisi* Fowler, 1934, considered a synonym of *T. vittata*. Non-native populations are likely due to release from aquarium specimens.

Key words

DNA barcode; COI; ornamental fish; invasive species; Bangladesh; Myanmar.

Academic editor: Zeehan Jaafar | Received 13 January 2017 | Accepted 16 May 2017 | Published 12 July 2017

Citation: Norén M, Kullander SO, Rahman MM, Mollah, AR (2017) First records of Croaking Gourami, *Trichopsis vittata* (Cuvier, 1831) (Teleostei: Osphronemidae), from Myanmar and Bangladesh. Check List 13 (4): 81–85. <https://doi.org/10.15560/13.4.81>

Introduction

Trichopsis vittata (Cuvier, 1831), known as Croaking Gourami in the ornamental fish trade, is a small air-breathing osphronemid fish, reported as native from Java, Borneo, Sumatra, Peninsular Malaysia, Thailand, and the Mekong basin in Cambodia, Laos, and Vietnam (Kottelat 1985, Rainboth 1996, Baird et al. 1999). *Trichopsis vittata* is found in shallow, slow-flowing or stagnant waters with abundant vegetation, such as rice fields, roadside ditches, and irrigation canals. It is able to survive in brackish water up to 20 psu salinity, and in temperatures down to 7.2 °C (Schofield and Schulte 2016). It feeds on small planktonic crustaceans and insect larvae (Rainboth 1996). The male is territorial and builds

a small inconspicuous bubble nest among the vegetation, in which the eggs are deposited and guarded by the male until the larvae become free-swimming (Britz et al. 2001, Liengpornpan et al. 2006). Due to its small size there is no targeted fishery for *T. vittata*, but it is occasionally sold in markets as part of mixed catches, and is regularly seen in the ornamental fish trade (Rainboth 1996).

The FAO *Introduction of Species* database lists *T. vittata* as introduced only to the USA, where a feral population has persisted in southern Florida since the 1970s (Lee et al. 1980, Schofield and Pecora 2013), but established feral populations of *T. vittata* have also been reported from the Philippines (BFAR 2006), without supporting data, and most recently from Chennai in Tamil Nadu, India (Knight and Balasubramanian 2015). Here

we present additional localities from Myanmar and Bangladesh, up to 1000 km west of the natural range of the species and 1500 km northeast of the previously known feral population in Chennai, and discuss the origin and future development of feral *T. vittata* in South Asia.

Methods

All vouchered localities in Myanmar and Bangladesh are presented in Table 1 and mapped in Figure 1. All listed specimens were collected using beach seine or handheld nets, or purchased at fish markets, and morphologically identified. The standard barcoding region of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was sequenced from 5 *T. vittata* collected in Bangladesh, 6 collected in Myanmar, and 3 aquarium specimens (GenBank accession numbers: KT250366, KY327366, KY327367) purchased in Sweden, using the primers and protocol of Ward et al. (2005).

The obtained DNA sequences were assembled in Geneious version 9 (KEARSE et al. 2012), and combined with all 134 COI sequences identified as *T. vittata*, *T. cf. vittata*, or *T. sp. cf. vittata* deposited on GenBank, to produce a matrix from which to estimate sequence divergence as *pairwise p-distance*, the percentage of differing nucleotide sites between 2 compared DNA sequences (please note that this is a simple measure of dissimilarity and different from the similarly-named statistical term *p-value*). A neighbor-joining majority rule bootstrap tree (Jukes-Cantor model, 500 repetitions, random addition sequence) rooted with *Betta macrostoma* (GenBank Accession number KM485437) was produced to visualize genetic similarity (not shown) The *Identification* tool of the Barcode of Life portal (<http://boldsystems.org>) was used to obtain the Barcode of Life Barcode Index Numbers (BINs) of clusters of similar published sequences, corresponding to putative species. Voucher specimens are deposited in the collections of Dhaka University and the Swedish Museum of Natural History.

Published occurrence data used to construct occurrence map (Fig. 2) were obtained from the Academy of Natural Sciences at Philadelphia, Auburn University Museum of Natural History, California Academy of Sciences, Field Museum, Florida Fish and Wildlife Conservation Commission, Illinois Natural History Survey, GBIF-MNHN (Paris), Oregon State University, Royal Ontario Museum, Florida Museum of Natural History, University of Michigan Museum of Zoology, United States National Museum of Natural History, Smithsonian Institution, Yale University Peabody Museum, and Natural History Museum of Denmark (Accessed through the Fishnet2 Portal, <http://www.fishnet2.net>, 2015-5-26).

Collecting was made with permission from the Department of Environment in Bangladesh to the Department of Zoology, University of Dhaka, and from the Department of Fisheries in Myanmar to Swedish teams.

Table 1. Voucher data for the non-native Croaking gourami (*Trichopsis vittata*) recorded from Myanmar and Bangladesh. NRM = Swedish Museum of Natural History; USNM = National Museum of Natural History, Smithsonian Institution (database record only).

Voucher numbers	n	Country	Genbank Acc#	River drainage	Locality	Lat. °N	Lon. °E	Date
NRM 39950	1	Myanmar		Salween	Roadside ditch at Zayitchaung village, on the road Kyaikto-Thaton, 5 km before Thaton	17.0131	97.3356	16 Mar. 1997
USNM 378943	6	Myanmar		Salween	Kawkaireik market	—	—	1 Jan. 2002
NRM 58106	1	Myanmar		Salween	Thathon market	16.9271	97.3621	17 Mar. 2008
NRM 58339	1	Myanmar		Salween	God Chaung, 2 miles on the way from Thathon to Pha-An	16.9086	97.3887	17 Mar. 2008
NRM 58410–58584, 67131	101	Myanmar	KT250368	Salween	Roadside ditch near bridge over Salween River at Tayoke Hla village, 5 miles to Pha-An	16.8331	97.6261	17 Mar. 2008
NRM 65183, 65291, 66324	12	Myanmar	KY327369	Ayeyarwaddy	Lake in small village, north of Nyaungdon, 68 km NW Yangon	17.1036	95.5895	24 Nov. 2013
NRM 65142, 65145, 65146, 66311–66312, 66395–66398	83	Myanmar	KY327368 KY327370	Yangon	Irrigation channel on road Yangon - Patheingyi, 25 km NW Yangon	16.9113	95.9441	24 Nov. 2013
NRM 67073	1	Myanmar	KY327372	Ayeyarwaddy	Small stream in village, alongside road, 3 km NE Pantanaw, 75 km WNW Yangon	16.9992	95.4884	24 Nov. 2013
NRM 67056–67058	3	Myanmar	KY327371	Ayeyarwaddy	Small stream in Kha Yaykan village, 9 km N Innma village, 95 km NW Yangon	17.1451	95.3312	25 Nov. 2013
NRM 66672–66674, 66676–66681, 67094–67095	65	Bangladesh	KY349109	Old Brahmaputra	Turag River at Kamarpore, near Dhaka city	23.8985	90.3844	1 Dec. 2014
NRM 66578–66581, 67096	12	Bangladesh	KT250365 KT250367	Old Brahmaputra	Fish market in Shonbari, Sreenagar	23.544	90.2964	2 Dec. 2014
NRM 69396, 69461	2	Bangladesh	KY327373 KY327374	Old Brahmaputra	Dhaleshwari River near Abdullahpur Bridge, Keranigonj	23.3850	90.2046	25 Feb. 2016
NRM 69220, 69434–69435	3	Bangladesh		Old Brahmaputra	Turag River at Ashulia point near Dhaka city	23.5135	90.2049	5 Mar. 2016
NRM 68345, 69493	3	Bangladesh		Meghna	Shorail, roadside ditch 8 km north of Brahmanbaria	24.0472	91.1026	19 Mar. 2016
NRM 68562	5	Bangladesh		Meghna	Titas River in Akhaura	23.884	91.2012	19 Mar. 2016

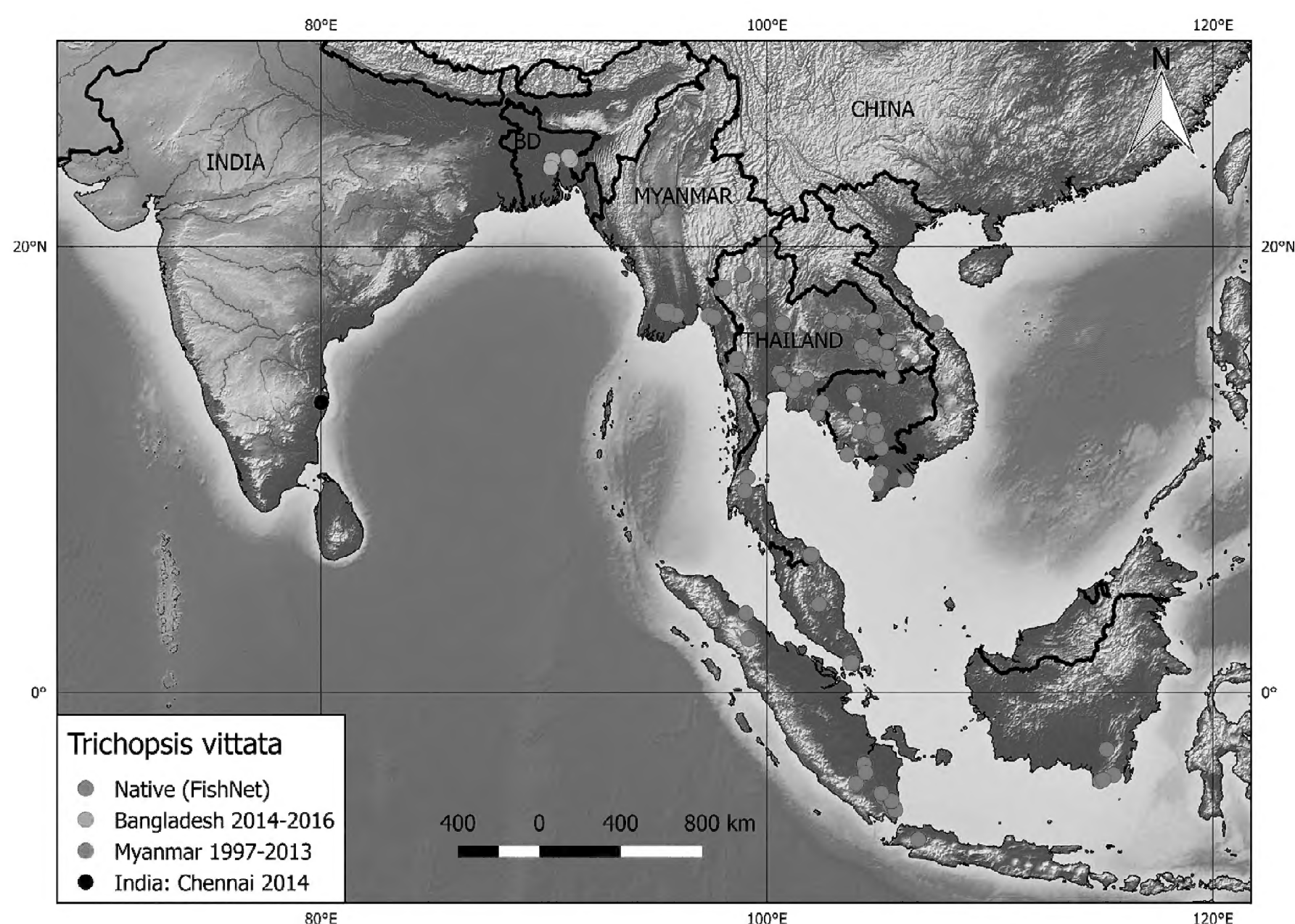


Figure 1. Distribution of *Trichopsis vittata* in Asia, based on occurrence records in FishNet II (1906–2014), Knight and Balasubramanian (2015), and University of Dhaka and Swedish Museum of Natural History records (Myanmar 1997–2013; Bangladesh 2014, 2016). BD = Bangladesh.

Results

Trichopsis vittata can be separated from the 2 other currently recognized species in the genus, *Trichopsis pumila* (Arnold, 1936) and *Trichopsis schalleri* Ladiges, 1962, by the presence of a thin dark line below the eye. *Trichopsis vittata* also grows to a larger maximum size, and normally has 3 longitudinal dark bands on the posterior half of the body instead of 2 as in *T. schalleri* and *T. pumila* (in *T. pumila* the upper of the 2 bands is broken into a row of dark spots surrounded by iridescent scales) (Fig. 2). Rainboth (1996) states that *T. vittata* grows up to 70 mm standard length (SL). Sithtananan (2010) measured 848 individuals from all over the native distribution and found a maximum length of 48.8 mm SL, and average length 31 mm SL.

Discussion

There are no published records of *T. vittata* from Bangladesh or Myanmar previous to the present report, not even from the Myanmar portion of the Mekong River, but the species has been common in collections from Indochina and Indonesia for more than a century, suggesting that the Tenasserim and Sino-Burman ranges separating the Indochinese watersheds from the Burmese have been a barrier to westward dispersal (Fig. 1). In 1997, staff from the Swedish Museum of Natural History collected a single *T. vittata* from a roadside ditch 5 km north of the town of Thaton, in Mon State in eastern Myanmar.

The individual was a juvenile, showing that the species was already reproducing in the region. In 2008, numerous specimens were collected at different localities in Kayin State in eastern Myanmar, and in 2013 we found numerous specimens at different localities in southern

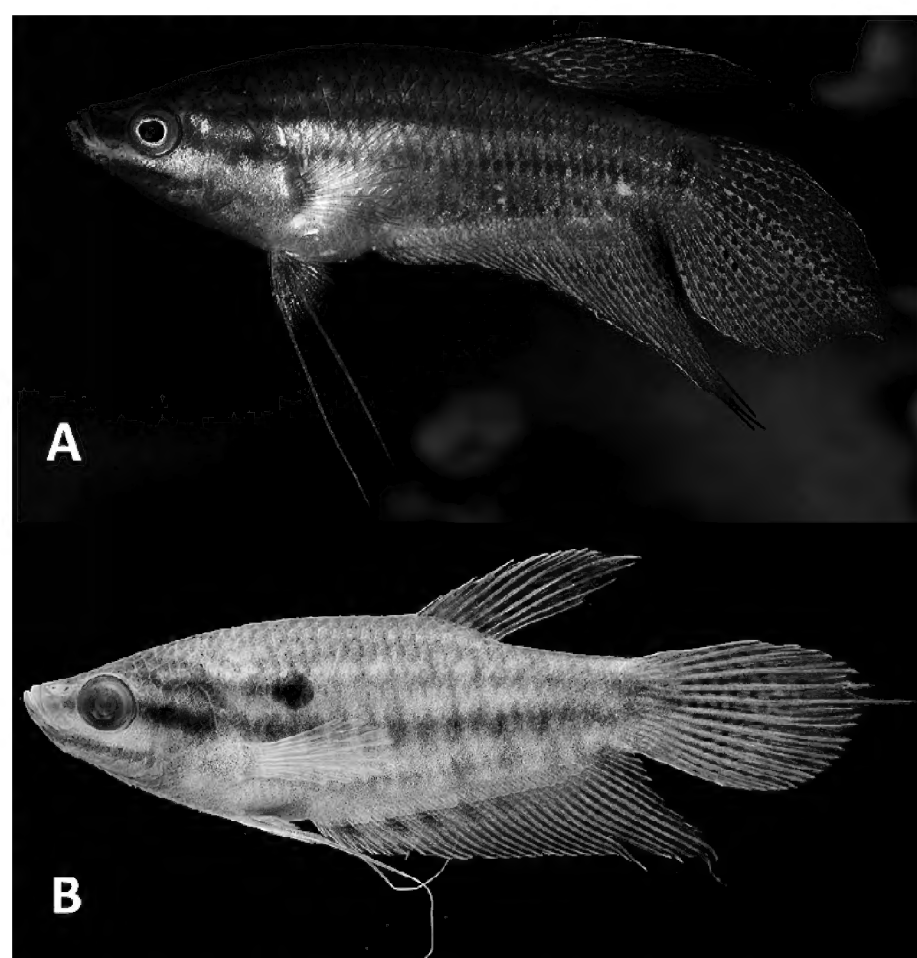


Figure 2. *Trichopsis vittata*. **A.** Live male specimen photographed in aquarium (not preserved), **B.** Preserved specimen (NRM67094c), 32.8 mm SL, collected from Turag River, Bangladesh, 2014. The dark pigmented line below the eye is a diagnostic character for *T. vittata*.

Myanmar west of the city of Yangon, and additional specimens were obtained in the Kawkaeik district in eastern Myanmar by Mr. U Tin Win. The earliest records of *T. vittata* from Myanmar are from the eastern part of the country, but no collections are available from the same time in western Myanmar, so the dispersal within Myanmar should not be assumed to have been from east to west. In 2014 we found the species in abundance in the Turag River, a tributary of the lower Brahmaputra flowing through Dhaka, and at the fish market in the nearby Sreenagar (Dhaka Division) in central Bangladesh (Table 1). The species was recorded again in 2016 from the Turag River, and also in the nearby Dhaleswari River as well as 2 localities in the lower Meghna River northeast of Dhaka (Table 1). No *T. vittata* were found in southeastern Bangladesh (Chittagong Division) during a survey in 2015, or in northeastern Bangladesh (Sylhet Division), surveyed in 2016, despite the presence of suitable habitat.

Panijpan et al. (2015) analyzed mitochondrial COI and nuclear RAG1 DNA sequences of a large number of *Trichopsis* from Southeast Asia, and found that *T. vittata* is not a single species but a species complex comprising 4 ecologically and morphologically similar but genetically distinct biological species. However, there were no DNA sequences available from Java, the type locality of *T. vittata* (Roberts 1996), and Panijpan et al. (2015) were unable to determine which, if any, of these 4 clades represented the true *T. vittata*. Dahrudin et al. (2016) published 6 sequences of *T. vittata* from Java, which in our analysis correspond to the clade that Panijpan et al. (2015) referred to as “*Trichopsis* cf. *vittata* 4”, from southern Thailand (BIN cluster ID BOLD:AAB9368).

As a coarse empirical rule-of-thumb, for the COI gene, the dissimilarity between any 2 members of the same species is typically $< 1\%$, while the dissimilarity between members of different species is typically $\geq 2\%$ (Ward 2009). This disjunct distribution of genetic similarity has been termed “the barcode gap” (Meyer and Paulay 2005), and can be used as an indicator of species affinity through a so-called barcode analysis, where a DNA sequence from an unidentified specimen is compared to a database of corresponding DNA sequences from well-identified specimens, allowing confident identification also of single unknown sequences.

The COI sequences of *T. vittata* from Myanmar, Bangladesh, and the ornamental fish trade are similar ($\leq 0.6\%$ *p*-distance) to each other and to 78 published COI sequences, corresponding to a clade Panijpan et al. (2015) referred to as “*Trichopsis* sp. (cf. *vittata*) 3” (BIN cluster ID BOLD:ACS9775), from western and central Thailand, but dissimilar ($> 2.4\%$ *p*-distance) to all individuals of *T. vittata* from other areas. This supports the conclusion by Panijpan et al. (2015) that the current *T. vittata* is a species complex, and indicates that *T. vittata* sold in the ornamental fish trade in Europe and the introduced populations in Bangladesh and Myanmar all originates from central and western Thailand. According to Panijpan et al. (2015), “*Trichopsis* sp. (cf. *vittata*) 3” is

distributed in Thailand from Chiang Mai in the northwest, to Trat in the southeast, and Surat Thani in the south. Fowler (1934) described *T. harrisi* from Kratt (Trat), but Kottelat (2001) considered *T. harrisi* a junior synonym of *T. vittata*. A taxonomic revision of *Trichopsis* is needed, and may come to the conclusion that the correct name for “*Trichopsis* sp. (cf. *vittata*) 3” is *T. harrisi*.

The probable source of the feral population of *T. vittata* in Florida is a nearby ornamental fish farm, and also in the other locations the presumed source is release of ornamental fish (Schofield and Pecora 2013, Knight and Balasubramanian 2015). However, while Thailand is a major exporter of ornamental fish, and *T. vittata* does occur in the ornamental fish trade, it is not common. Pygmy gourami (*T. pumila*) is the most common species of *Trichopsis* in the ornamental fish trade, but feral populations have only been reported from the Philippines (BFAR 2006), without data. This suggests either that *T. vittata* is able to colonize new territory even when propagule size and frequency are low, or that it spreads also through some mechanism other than release of aquarium fish, such as intentional release for biological control of mosquitos or as a contaminant among shipments of live fish for aquaculture.

Wongsiri (1982) found that in Thailand the 3 most important fish predators of mosquito larvae were *T. vittata*, Guppy (*Poecilia reticulata* Peters, 1859) and Tilapia (*Oreochromis* sp.). The ability of *T. vittata* to survive in very small volumes of water, and to tolerate polluted or low-oxygen water, could make it suitable for mosquito control, but we can find no record of it having been intentionally released as a mosquito control agent.

Another possible avenue for introduction is as a contaminant in shipments of live aquaculture fish. The small East Asian Topmouth Gudgeon, *Pseudorasbora parva* (Temminck & Schlegel, 1846), was introduced to Eastern Europe as a contaminant with fingerlings of Chinese cyprinids imported for aquaculture trials and has since spread to many river systems in Europe (Gavriloaie et al. 2008). In Asia, *T. vittata* could spread as a contaminant in shipments of for instance live Silver Barb, *Barbonymus gonionotus* (Bleeker, 1849), native to Southeast Asia, sympatric with *T. vittata*, and an important aquaculture species in South and Southeast Asia (Gupta and Rab 1994), but both Myanmar and Bangladesh have a range of cultured species (Siddiqui et al. 2007) which present similar potential. Nevertheless, the records from India, Myanmar and Bangladesh are close to major cities, where aquarium releases are more likely, and in the absence of evidence that *T. vittata* spreads as a contaminant, and considering that the feral populations are genetically similar to specimens from the aquarium trade, we consider the release of specimens from the aquarium trade the most probable mechanism of introduction.

Trichopsis vittata has many of the traits typical of efficient invaders: it is able to tolerate polluted or low oxygen conditions, is human-associated (through aquaria, rice fields, and its ability to survive in degraded habitats),

has a short generation time, guards its eggs (uniparental care), and has a prior history of successful invasions. The preferred habitat of *T. vittata* (shallow, often temporary, vegetated waters with seasonal floods) corresponds to all areas suitable for rice-production, suggesting that it could potentially colonize most of southern and eastern Asia. However, *T. vittata* does not have any mechanism for long-distance dispersal, as it is non-migratory and does not have planktonic eggs or larvae, and non-human mediated spread is likely to be slow. In addition, it may locally be limited by life-history variables or interactions with the native biota, as seems to be the case with the population in Florida (Schofield and Schulte 2016).

No negative effects resulting from introduction of *T. vittata* have been reported, but Knight and Balasubramanian (2015) speculated that in India it may compete for niche space with native small osphronemids such as Dwarf Gourami [*Trichogaster lalius* (Hamilton, 1822)] and Spiketail Paradisefish [*Pseudosphromenus cupanus* (Cuvier, 1831)]. Other potential negative effects include aggressive displacement of native species, or that it may act as a vector for non-native parasites or pathogens, such as the trematode *Euclinostomum heterostomum* (Rudolphi, 1809) (Purivirojkul and Sumontha 2013).

Acknowledgements

This study is part of the project “Genetic characterization of freshwater fishes in Bangladesh using DNA barcodes” (Swedish Research Council, contract D0674001 to Sven Kullander and Abdur Rob Mollah).

Authors’ Contributions

All authors contributed to collection and text. MN and MR extracted and sequenced DNA. MN made the analyses. SOK made images and table.

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